

# CRISPR-based herd immunity can limit phage epidemics in bacterial populations

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**Abstract** Herd immunity, a process in which resistant individuals limit the spread of a pathogen among susceptible hosts has been extensively studied in eukaryotes. Even though bacteria have evolved multiple immune systems against their phage pathogens, herd immunity in bacteria remains unexplored. Here we experimentally demonstrate that herd immunity arises during phage epidemics in structured and unstructured *Escherichia coli* populations consisting of differing frequencies of susceptible and resistant cells harboring CRISPR immunity. In addition, we develop a mathematical model that quantifies how herd immunity is affected by spatial population structure, bacterial growth rate, and phage replication rate. Using our model we infer a general epidemiological rule describing the relative speed of an epidemic in partially resistant spatially structured populations. Our experimental and theoretical findings indicate that herd immunity may be important in bacterial communities, allowing for stable coexistence of bacteria and their phages and the maintenance of polymorphism in bacterial immunity.

## Introduction

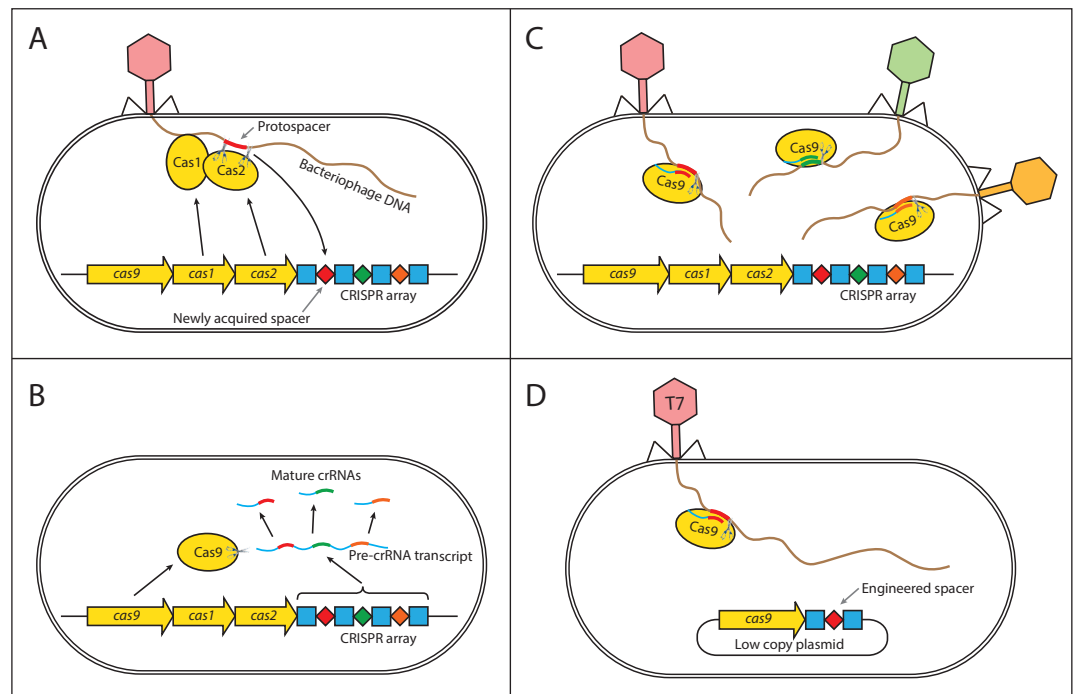
The term “herd immunity” has been used in a variety of ways by different authors (see *Fine et al., 2011*). Here, we define it as a phenomenon where a fraction of resistant individuals in a population reduces the probability of transmission of a pathogen among the susceptible individuals. Furthermore, if the fraction of resistant individuals in a population is sufficiently large the spread of a pathogen is suppressed. Experimental research into the phenomenon has focused mostly on mammals (*Jeltsch et al., 1997; Mariner et al., 2012*), birds (*Boven et al., 2008; Meister et al., 2008*), and invertebrates (*Konrad et al., 2012; Wang et al., 2013*). In human populations the principles of herd immunity were employed to limit epidemics of pathogens through vaccination programs (*Fine et al., 2011*), which in the case of smallpox lead to its eradication between 1959 and 1977 (*Fenner, 1993*).

Alongside advances in vaccination programs, the formalization of a general theory of herd immunity was developed. The theory is based on a central parameter,  $R_0$ , which describes the fitness of the pathogen, as measured by the number of subsequent cases that arise from one infected individual in a population (for a historical review of  $R_0$  see (*Heesterbeek, 2002*)). Thus,  $R_0$  indicates the epidemic spreading potential in a population. Given  $R_0$  the herd immunity threshold is defined as,

$$H = \frac{R_0 - 1}{R_0}, \quad (1)$$

which determines the required minimum fraction of resistant individuals needed to halt the spread of an epidemic.  $R_0$  and subsequently also  $H$  are affected by the specific details of transmission and the contact rate among individuals (*Grassly and Fraser, 2008*). Many theoretical studies have addressed the influence of some of these details, in particular maternal immunity (*Anderson and May, 1992*), age at vaccination (*Anderson and May, 1982; Nokes and Anderson, 1988*), age related or seasonal differences in contact rates (*Schenzle, 1984; Anderson and May, 1985; Yorke et al., 1979*), social structure (*Fox et al., 1971*), geographic heterogeneity (*Anderson and May, 1984; Lloyd and May, 1996; Real and Biek, 2007*), and the underlying contact network of individuals (*Ferrari et al., 2006*).

Interestingly, little work has focused on the potential role of herd immunity in microbial systems which contain a number of immune defense systems and have an abundance of phage pathogens. These defenses vary in their potential to provide herd immunity as they target various stages of the



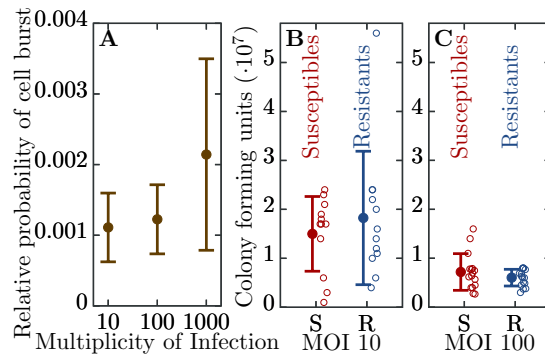
**Figure 1. Mechanism of CRISPR/Cas type II immunity.** The CRISPR/Cas system provides immunity to phages and its main features can be described by three distinct stages. (A) Acquisition. When a cell gets infected by a phage, a protospacer on the invading phage DNA (indicated as a red bar) is recognized by Cas1 and Cas2. The protospacer is cleaved out and ligated to the leader end (proximal to the Cas genes) of the CRISPR array as a newly acquired spacer (red diamond). (B) Processing. The CRISPR array is transcribed as a Pre-crRNA and processed by Cas9 (assisted by RNaseIII and trans-activating RNA, not shown) into mature crRNAs. (C) Interference. Mature crRNAs associate with Cas9 proteins to form interference complexes which are guided by sequence complementarity between the crRNAs and protospacers to cleave invading DNA of phages whose protospacers have been previously incorporated into the CRISPR array. (D) A truncated version of the CRISPR system on a low copy plasmid, which was used in this study lacks *cas1* and *cas2* genes and was engineered to target a protospacer on the T7 phage chromosome to provide *Escherichia coli* cells with immunity to the phage. The susceptible strain contains the same plasmid except the spacer does not target the T7 phage chromosome.

51 phage life cycle, from adsorption to replication and lysis. Early defense mechanisms include the  
 52 prevention of phage adsorption by blocking of phage receptors (Nordström and Forsgren, 1974),  
 53 production of an extracellular matrix (Hammad, 1998; Sutherland et al., 2004), or the excretion of  
 54 competitive inhibitors (Destoumieux-Garzon et al., 2005). Alongside these bacteria have evolved  
 55 innate immune systems that target phage genomes for destruction. These include host restriction-  
 56 modification systems (RMS) (Blumenthal and Cheng, 2002), argonaute-based RNAi-like systems  
 57 (Swarts et al., 2014), and bacteriophage-exclusion (BREX) systems (Goldfarb et al., 2015). In addition

to innate systems, bacteria have evolved an adaptive immune system called CRISPR-Cas (clustered regularly interspaced short palindromic repeat) (*Sorek et al., 2013*). In order for any of these immune systems to provide herd immunity, they must prevent further spread of the pathogen. Therefore, unless the phage particles degrade in the environment at a timescale comparable to the phage adsorption rate, the immune system must provide a ‘sink’ for the infectious particles reducing the average number of successful additional infections below one. Unlike the early defense mechanisms that may simply prevent an infection but not the further reproduction of infectious particles, the RMS, BREX, argonaute-based RNAi-like, and the CRISPR-Cas systems degrade foreign phage DNA after it is injected into the cell, and thus continue to remove phage particles from the environment, which increases their potential to provide herd immunity. In order for herd immunity to arise, the population must also be polymorphic for immunity, which can be achieved if immunity is plasmid borne. In addition to this, the CRISPR-Cas system is unique in that it is adaptive allowing cells to acquire immunity upon infection (see Fig 1A, B, and C), which can lead to polymorphism in immunity even if the system is chromosomal.

In addition to immune system-specific factors, the reproductive rate of phage depends strongly on the physiology of the host bacterium (*Hadas et al., 1997*), and the underlying effective contact network which may vary greatly in bacterial populations depending on the details of their habitat. Thus, herd immunity will be influenced by the physiological state of the bacteria and the mobility of the phage in the environment through passive diffusion and movement of infected individuals. Taken together these details call into question the applicability of the traditional models of herd immunity from vertebrates to phage-bacterial systems. Thus, experimental investigation and further development of extended models that take into account the specifics of microbial systems are required.

To investigate under which conditions herd immunity may arise in bacterial populations, we constructed an experimental system consisting of T7 phage and bacterial strains susceptible and resistant to it. Our experimental system can be characterized by the following features. First, we used two strains of *Escherichia coli*, one with an engineered CRISPR-based immunity to the T7 phage, and the other lacking it (Fig 1D). Second, we examined the dynamics of the phage spread in different environments – spatially structured and without structure. Furthermore, we developed and analyzed a spatially explicit model of our experimental system to determine the biologically relevant parameters necessary for bacterial populations to exhibit herd immunity.



**Figure 2. Efficiency of bacterial resistance.** (A) The probability that a resistant cell bursts, relative to a susceptible cell, at three different initial multiplicities of infection (MOI). The probability that a resistant cell bursts at MOI 1000 is significantly higher than at MOI 10 ( $p = 0.019$ ,  $t_4 = 3.031$ ) or at MOI 100 ( $p = 0.022$ ,  $t_5 = 2.674$ ). The error bars show the standard deviations from the mean. Note that this measure is not a widely used 'efficiency of plating' but it determines the probability of burst of single resistant cells (see Materials and Methods for details). (B) The number of colony forming units (CFUs) post phage challenge (see Materials and Methods). The mean number of CFUs after the bacterial cultures were exposed to the phage is not significantly different between susceptible and resistant strains at MOI 10 ( $p = 0.239$ ,  $t_{22} = 0.721$ ) and (C) at MOI 100 ( $p = 0.27$ ,  $t_{30} = 1.124$ ), indicating that the resistant cells' growth is halted after the cells are infected by a phage. The error bars show the standard deviations from the mean. There were no detectable CFUs in either susceptible or resistant cell cultures at MOI 1000. It should be noted that the indicated MOI values do not correspond to the average number of phages that adsorb to cells in the experiments. For MOI 10 we estimated the mean number of phages per cell as 0.229 and for MOI 100 as 0.988 (see Materials and Methods for details). It was impossible to determine the mean for MOI 1000 as there were no detectable CFUs under such conditions. The data presented in this figure can be found in Figure 2-source data 1.

The following source data are available for Figure 2:

Source data 1. Efficiency of bacterial resistance.

## Results

### Properties of resistant individuals

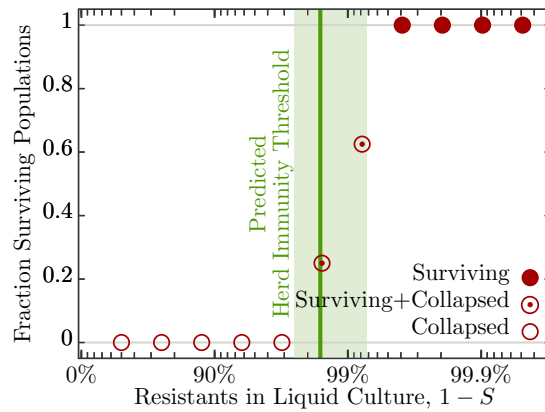
We engineered a resistant *E. coli* strain by introducing the CRISPR-Cas Type II system from *Streptococcus pyogenes* with a spacer targeting the T7 phage genome (see Material and Methods). We further characterized the ability of the system to confer resistance to the phage. We find a significant level of resistance as measured by the probability of cell burst when exposed to T7 (Fig 2A). However, resistance is not fully penetrant as approximately 1 in 1000 resistant cells succumb to infection. In addition, we observe that as phage load increases (multiplicity of infection, MOI) the probability that a cell bursts increases (Fig 2A). In order to determine the herd immunity threshold in our

experimental system, we constructed the resistant strain such that upon infection the cell growth is halted, yet the cell still adsorbs and degrades phages (Fig 2B,C). This feature is important as it prevents the action of frequency dependent selection which in naturally growing populations will favor the resistant strain until its frequency reaches the herd immunity threshold. Thus, in our system if the frequency of the resistant strain is below the herd immunity threshold, the resistant cells remain below the threshold and are unable to stop the epidemic and the whole population collapses. In contrast, if the frequency of resistant individuals in the population is above the herd immunity threshold, the resistant individuals provide complete herd immunity and the population survives. These properties allow us to quantify the expanding epidemic in both liquid media and on bacterial lawns (without and with spatial structure, respectively) using high throughput techniques. Specifically, it allows us to control for the complex dynamics of the system arising from frequency dependent selection and simultaneous changes in the physiological states of the cells (growth rates depending on the nutrient concentrations) and phage (burst size, latent period depending on the cell's physiology).

It should be noted that our model does not reflect this artificial property – it assumes that resistant bacteria keep growing after successfully overcoming a phage infection (see Eqn. (2d)). This discrepancy, however, does not affect the model prediction of the herd immunity threshold in our experimental system for the following reason: time scale of an epidemic spread through a population (double exponential phage growth) is substantially shorter than the time scale of bacterial population growth (exponential growth). Therefore, whether or not an epidemic is established does not depend on later dynamics of frequencies of resistant and susceptible individuals in the population, it only depends on the initial conditions. Similarly, the model correctly captures the dynamics of an epidemic in spatially structured populations as the phage spreads radially and in every time-point the epidemic front encounters a naive population with a constant ratio of resistant to susceptible individuals.

### **Herd immunity in populations without spatial structure**

To understand the influence of spatial population structure, or lack thereof, we first measured the probability of population survival (i.e., whether the cultures are cleared or not) in well mixed liquid environments (no spatial structure) consisting of differing proportions of resistant to susceptible individuals and T7 phage. When the percentage of resistant individuals is in excess of 99.6% all



**Figure 3. Fraction of surviving populations at 18 h post phage infection.** Bacterial populations consisting of various fractions of resistant to susceptible individuals infected with  $\approx 50$  phages, corresponding to a multiplicity of infection (MOI) of  $\approx 10^{-4}$ , designed to resemble an epidemic initiated by the burst size from one infected individual (see Table 3 for burst size estimates). Each population phage challenge is replicated 16 times. The solid dark green line shows the model prediction, Eqn. (4), for the herd immunity threshold ( $H$ ), given latent period ( $\lambda$ ), bacterial growth rate ( $\alpha$ ), and phage burst size ( $\beta$ ). Shaded area indicates  $\pm 1$  standard deviation. The data presented in this figure can be found in Figure 3–source data 1.

The following source data are available for Figure 3:

Source data 1. Fraction of surviving populations at 18h post phage infection.

16 replicate populations survive a phage epidemic (i.e., show no detectable difference in growth profiles to the phage free controls; Fig 3). Populations with 99.2% and 98.4% resistant individuals show intermediate probabilities of survival – 10 out of 16 replicate populations and 4 out of 16 replicate populations survive, respectively (Fig 3). The likely explanation as to why some populations survive and others collapse is due to the stochastic nature of phage adsorption after inoculation: If the population composition is close to the herd immunity threshold a stochastic excess of phage particles adsorbing to susceptible cells may trigger an epidemic, whereas if chance increases the number of phages adsorbing to resistant individuals, the epidemic is suppressed. However, when populations have fewer than 96.9% resistant individuals all 16 replicate populations fail to survive and collapse under the epidemic (Fig 3).

As mentioned in the introduction, phage and bacterial physiology may affect the herd immunity threshold. To test this we altered bacterial growth by reducing the concentration of nutrients in the medium by mixing LB broth with 1X M9 salts in different ratios (Fig 6), which concurrently alters the T7 phage's latent period and burst size (Fig 4A,B and Table 3). Indeed, we observe as bacterial growth rates decline the fraction of resistant individuals necessary for population survival

decreases (Fig 4C). When the populations are grown in a 50% diluted growth medium, the fraction of resistant individuals required for a 100% probability of survival is 99.2%; when the fraction of resistant individuals is 75% or less populations go extinct. In a 20% growth medium the fraction of resistant individuals required for survival decreases to 96.9%, while the fraction when all replicates collapse to 50%.

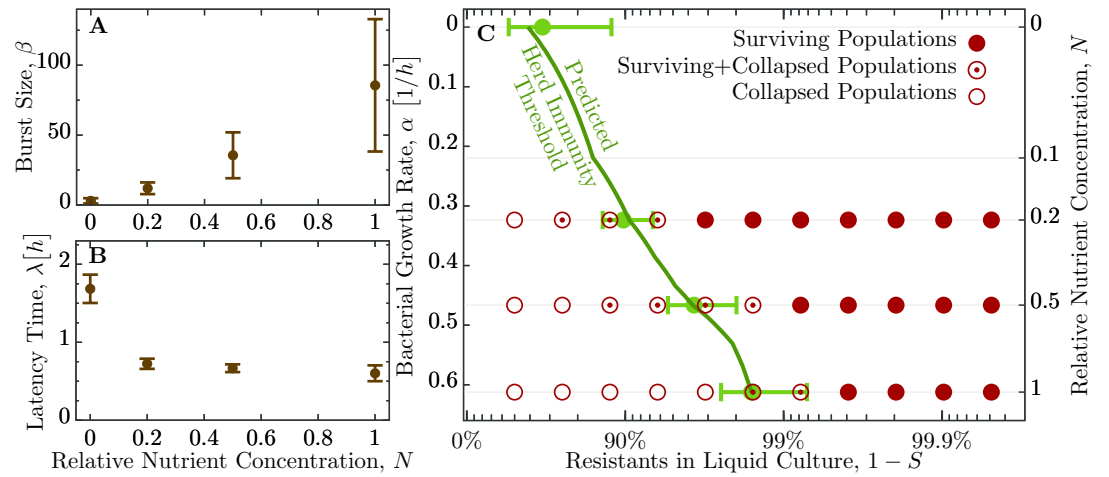
From the experimental observations of the herd immunity threshold values we infer the phage  $R_0$  using Eqn. 1. In an undiluted growth medium the phage  $R_0$  falls between 32 and 256 and decreases to between 4 and 128 in 50% and between 2 and 32 in 20% nutrient medium. These data indicate that bacterial populations can exhibit herd immunity in homogeneous liquid environments. However, bacteria typically live in spatially structured environments such as surfaces, biofilms or micro-colonies, therefore we extended our experiments to consider the potential impact of spatially structured populations.

### **Herd immunity in spatially structured populations**

In order to discern the role, if any, spatial structure plays in herd immunity we conducted a set of experiments in spatially structured bacterial lawns on agar plates. Spatially structured bacterial populations provide a more fine grained measure of herd immunity, compared to the population survival assays done in liquid culture. On bacterial lawns, phages spread radially from a single infectious phage particle and the radius of plaque growth on different proportions of resistant to susceptible individuals can be easily quantified. In addition, these data allow for estimating the speed of the epidemic wave front in these different regimes using real-time imaging (Fig 5A).

We observe a decline in the number of plaque forming units (see Appendix figure 2) and a significant decrease in final plaque sizes as the proportion of resistant individuals in the populations increases (Fig 5B,C). A reduction in the final plaque size compared to a fully susceptible population was statistically significant with as few as 10% resistant individuals in a population ( $p = 0.004$ ,  $t_{53} = 2.744$ ). In order to determine the effect of resistant individuals during the earlier phase of bacterial growth (until the bacterial density on the agar plate reaches saturation; Fig 6A), we analyze the velocities of plaque growth between 0 and 24 hours post inoculation (*hpi*). We find that the speed is significantly reduced after 11 *hpi* when the population consists of as few as 10% of resistant individuals ( $p = 0.0317$ ,  $t_{32} = 1.923$ ). As the fraction of resistant individuals further increases, the speed declines significantly at earlier and earlier time points: 6 *hpi* with 20% ( $p = 0.0392$ ,  $t_{62} = 1.79$ ),





**Figure 4. Herd immunity threshold in liquid culture as a function of bacterial growth.** (A) Phage burst size ( $\beta$ ) change as a function of nutrient concentrations. (B) Latent period ( $\lambda$ ) increase across the range of nutrient concentrations. Values for  $\beta$  and  $\lambda$  are given in Table 3. Nutrient concentration  $N$  is measured relative to the standard protocol for LB medium. (C) Population survival analysis upon phage challenge as a function of the fraction of resistant cells and the intrinsic growth rate (nutrient availability,  $N$ ). Bacteria survive the phage infection (full circles), collapse (empty circles), or exhibit both outcomes (circled dots) in the 16 to 18 replicates, done in 3 independent batches. Light green errorbars for measured data show the expected value and its standard deviation of  $H(\alpha)$ , Eq. (5), with standard error propagation of the measured  $\beta$ ,  $\lambda$  and  $\alpha$ . In order to interpolate herd immunity to concentrations  $N$  not probed in experiments (dark green line), we use a second order polynomial in  $N$  to fit the data for both  $\beta/\lambda$  and  $\beta$ , which excellently matches the average measurements (a naive linear fit displays non-negligible deviations and non-sensical negative values). In addition, the dependence  $\alpha = \alpha(N)$  is obtained by numerically inverting the Monod growth rate dependence, see Eq. (9). The data presented in this figure can be found in Figure 4–source data1 and Figure 4–source data 2.

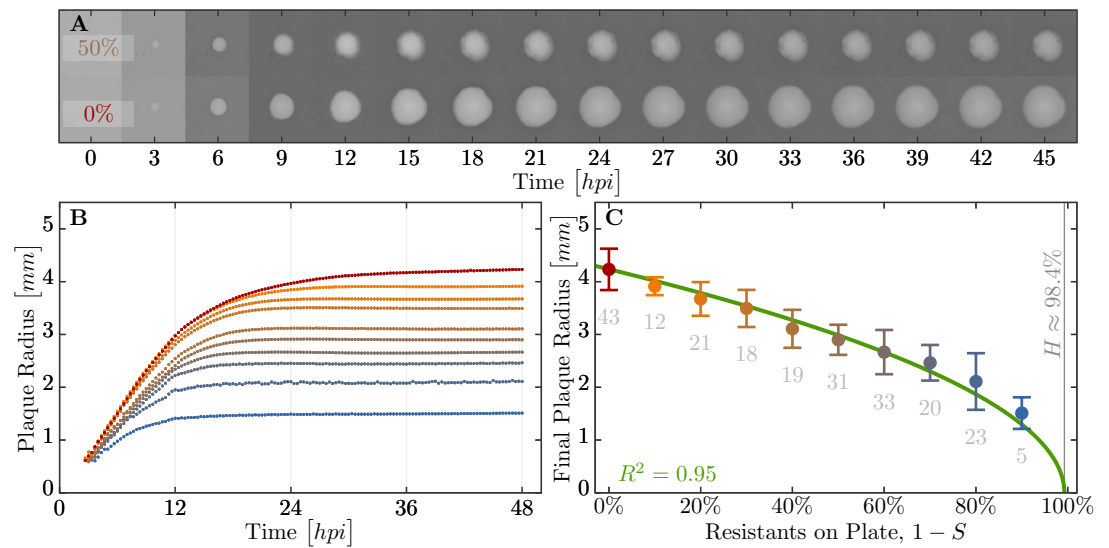
The following source data are available for Figure 4:

Source data 1. Figure 4A and 4B source data: Phage burst sizes and latent period in different dilutions of the growth medium.

Source data 2. Figure 4C: Fraction of surviving populations in different dilutions of the growth medium.

and 5.67 hpi with 30% ( $p = 0.0286$ ,  $t_{53} = 1.943$ ). In fact, when the fraction of resistant individuals exceeds 40%, the reduction in the speed of the spread is statistically significant immediately after the plaques are visually detectable (Fig 7). It should be noted that all populations with such low percentages of resistant individuals in liquid environment collapsed, indicating that spatial structure plays a role in herd immunity.

The results presented in this and the previous section would allow us to use Eq. (1) to infer a value for  $R_0$  from the observed threshold between surviving and collapsing bacterial populations,



**Figure 5. Properties of expanding phage epidemics on bacterial lawns.** (A) Example of plaque morphology and size change over 48 hours for populations with 50% resistant cells (top) and a control with 100% susceptible cells (bottom). (B) Mean plaque size area through time. Colors indicate the different fraction of resistant individuals (color coding as in panel C). Note the distinct two phases of plaque growth – initially, phage grow fast with exponentially growing bacteria but slow once the nutrients are depleted ( $\approx 10$  hours). The plaque radius is reduced, relative to 100% susceptible population, even when only a small fraction of resistant individuals are in the population. (C) Final plaque radius at 48 hpi. Green line shows the prediction from the model for the plaque radius  $r$ . Grey numbers indicate the number of plaques measured. Error bars indicate the standard deviations. The data presented in this figure can be found in Figure 5–source data1.

The following source data are available for Figure 5:

Source data 1. Plaque radii for all population compositions and time points.

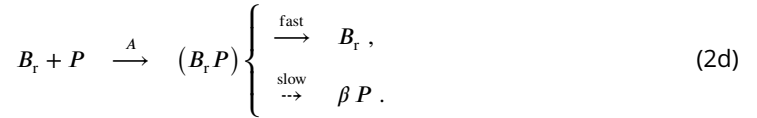
180 Figs 3 and 4. We also observe that herd immunity is strongly influenced by spatial organization of  
 181 the population, Fig 5. How the exact value of  $H$  (and subsequently the “classical” epidemiological  
 182 parameter  $R_0$ ) is affected by various factors such as bacterial growth rate, phage burst size and  
 183 latent period is, however, difficult to resolve experimentally. Similarly, quantification of the effect of  
 184 spatial structure is hardly achievable solely by experimental investigation. In order to disentangle  
 185 the roles of all the factors mentioned above, we proceed with development and analysis of a  
 186 mathematical model of the experimental system.

### 187 Modelling bacterial herd immunity

188 We developed a model of phage growth that takes several physiological processes into account:  
 189 bacterial growth during the experiment, bacterial mortality due to phage infection, and phage

190 mortality due to bacterial immunity. Furthermore, we use the previously reported observation that  
 191 phage burst size  $\beta$  and latent period  $\lambda$  depend strongly on the bacterial growth rate  $\alpha$  (see Table 2).

192 The main processes in our model system can be defined by the following set of reactions,



193 Susceptible ( $B_s$ ) and resistant ( $B_r$ ) cells grow at a rate  $\alpha$  (no significant difference in growth rate  
 194 between strains,  $\alpha(B_s) = 0.551 \pm 0.045 \text{ h}^{-1}$ ,  $\alpha(B_r) = 0.535 \pm 0.023 \text{ h}^{-1}$ ,  $t_{70} = 1.867$ ,  $p = 0.066$ ), (2a) and  
 195 (2b), by using an amount  $y$  of the nutrients  $N$ . Phage infection first involves adsorption to host cells,  
 196 (2c) and (2d), with the adsorption term  $A$  specified below. Infected susceptible bacteria produce on  
 197 average  $\beta$  phage with a rate inversely proportional to the average latency  $\lambda$ . In contrast, resistant  
 198 bacteria either survive by restricting phage DNA via their CRISPR-Cas immune system or – less likely  
 199 – succumb to the phage infection. However, when the MOI is large even resistant cells become  
 200 susceptible to lysis resulting in the release of phage progeny (see Fig 2) (*Westra et al., 2015; Chabas*  
 201 *et al., 2016*).

202 In our system, bacteria eventually deplete the available nutrients,  $N(t > T_{\text{depl}}) = 0$ , resulting in  
 203 the cessation of growth. This decline in bacterial growth affects phage growth – latency increases  
 204 and burst size decreases, such that phage reproduction declines dramatically (see Table 3). We  
 205 define the critical time point at which cells transition from exponential growth to stationary phase  
 206 as,

$$T_{\text{depl}} \approx \frac{1}{\alpha} \log \left( \frac{B_{\infty}}{B_0} \right). \quad (3)$$

207 Here,  $B_0$  and  $B_{\infty}$  are the initial and final bacterial densities, respectively. In the initial exponential  
 208 growth phase, our estimates from experimental data for growth parameters are  $\alpha = 0.63 \text{ h}^{-1}$ ,  $\beta =$   
 209  $85.6 \text{ phages/cell}$  and  $\lambda = 0.60 \text{ h}$ , for bacteria and phages, respectively (Table 2 and Table 3). After time  
 210  $T_{\text{depl}}$ , bacterial growth rate is set to zero ( $\alpha = 0$ ) and phage growth is reduced to  $\beta_{\text{depl}} = 3.0 \text{ phages/cell}$   
 211 and  $\lambda_{\text{depl}} = 1.69 \text{ h}$ . Such a two state model – constant growth rate while nutrients are present and no  
 212 growth after depletion – describes the observed population trajectories in experiments sufficiently  
 213 well (see Fig 6).

## Modelling herd immunity in populations without structure

An important parameter for estimating herd immunity is the fraction  $S$  of susceptible bacteria in the population. As a first estimate, a phage infection spreads in well mixed bacterial cultures if  $\beta S > 1$ , which leads to a continuous chain of infections: the product of burst size  $\beta$  of phage particles and the probability  $S$  of infecting a susceptible host has to be larger than one. As a first approximation, one could identify  $R_0$  with the burst size  $\beta$ , which is compatible with the observed herd immunity thresholds when inverting Eq. (1).

However, the growing bacterial population could outgrow the phage population if the former reproduces faster (e.g., in the case of RNA coliphages, *van Duin, 1988*), which introduces deviations from the simple relation between  $R_0$  and  $H$  as shown in (1). We capture this dynamical effect in a correction to the previous estimate as  $\beta S > 1 + \lambda\alpha$  (see Materials and Methods): more phages have to be produced for the chain of infections to persist in growing populations. The correction  $\frac{\lambda}{1/\alpha}$  is the ratio of generation times of phages over bacteria – usually, such a correction is very small for non-microbial hosts and can be neglected. Ultimately, herd immunity is achieved if the threshold defined by  $H = 1 - S_c$  is exceeded, with  $S_c$  the critical value in the inequality above. Rearranging, we obtain an expression for the herd immunity threshold

$$H = \frac{\beta - 1 - \lambda\alpha}{\beta} . \quad (4)$$

This estimate of  $H$  coincides to a very good extent with the population compositions of susceptible and resistant bacteria where we observe the transition from surviving and collapsed populations in experiments (see Fig 3). Moreover, simulations presented in the Appendix (section Simulation of recovery rate) show a range in the bacterial population composition with non-monotonic trajectories for  $B_s$  and  $B_r$  (see Appendix figure 1B), which is comparable to the range in composition we find in both outcomes, i.e., some surviving and some collapsing populations in experiments. For such parameter choices, stochastic effects could then decide the observed fates of bacteria. As presented above, the herd immunity threshold changes when the bacterial cultures grow in a diluted growth medium. In a set of independent experiments we measured bacterial growth rate  $\alpha$ , phage burst size  $\beta$  and phage latent period  $\lambda$  under such conditions (see Fig 6B and Table 3). From these data we estimated the dependence of the phage burst size on the bacterial growth rate,  $\beta(\alpha)$ , using a numerical quadratic fit (Fig 4A). Similarly, we estimated the dependence of the phage latent period on the bacterial growth rate,  $\lambda(\alpha)$  (Fig 4B). Using these estimates we calculated the expected growth

243 rate-dependent herd immunity threshold

$$H(\alpha) = \frac{\beta(\alpha) - 1 - \lambda(\alpha)\alpha}{\beta(\alpha)}, \quad (5)$$

244 which gives a very good prediction of the shift in the herd immunity threshold to lower values  
 245 for slower growing populations (green line in Fig 4C). This verification of our model shows that it  
 246 correctly captures the dependence of the herd immunity threshold on bacterial and phage growth  
 247 parameters.

248 The deviations from the herd immunity threshold depicted by the green area in Fig 3 and green  
 249 error bars in Fig 4C are derived from uncertainty in measurements in  $\beta$ ,  $\lambda$  and  $\alpha$ . The inherent  
 250 stochasticity of the adsorption process thus provides additional uncertainty, which is not captured  
 251 by the depicted error bars. This additional stochasticity can explain wider transition zone in  
 252 experiments with slower growing populations ( $N = 0.5$  and  $0.2$ ), because the fate of the population  
 253 is more prone to stochastic effects as the phage replication rate is slower than in a fast growing  
 254 population. This stochastic effect might be reduced by larger phage inocula. This could, however,  
 255 also shift the observed transition between collapsing and surviving populations towards higher  
 256 frequencies of resistant bacteria (and away from the actual herd immunity threshold) as protection  
 257 by the immune system is less effective with increasing number of phages per cell (see Fig 2A).

### 258 **Modelling herd immunity in spatially structured populations**

259 The dynamics of phage spread differ between growth in unstructured (e.g., liquid) and structured  
 260 (e.g., plates) populations. In order to quantify the effect of spatial structure in a population, we  
 261 extend our model to include a spatial dimension. In structured populations growth is a radial  
 262 expansion of phages defined by the plaque radius  $r$  and the expansion speed  $v$ , for which several  
 263 authors have previously derived predictions (*Kaplan et al., 1981; Yin and McCaskill, 1992; You and*  
 264 *Yin, 1999; Fort and Méndez, 2002a; Ortega-Cejas et al., 2004; Abedon and Culler, 2007; Mitarai*  
 265 *et al., 2016*).

266 We assume phage movement can be captured by a diffusion process characterized with a  
 267 diffusion constant  $D$ , which we estimate in independent experiments as  $D = 1.17 (\pm 0.26) \cdot 10^{-2} \text{ mm}^2/\text{h}$   
 268 (see Materials and Methods, Fig 8). However, we assume that only phages disperse and bacteria are  
 269 immobile as the rate of bacterial diffusion does not influence the expanding plaque on timescales  
 270 relevant in the experiment. Adsorption of phages on bacteria is modeled with an adsorption  
 271 constant  $\delta^*$ .

272 Taking these considerations together, allows to write a reaction-diffusion dynamics for growth  
273 of phages  $P$  on the growing bacterial population as

$$\partial_t P = D \partial_x^2 P + \delta^* (\beta S - 1 - \lambda \alpha) P . \quad (6)$$

274 The first term accounts for the diffusive spread of phages, while the second term describes phage  
275 growth. This second term includes the correction  $\lambda \alpha$  which arises due reproduction of bacteria,  
276 derived in the unstructured liquid case.

277 The spreading infection will sweep across the bacterial lawn with the following speed

$$v = 2 \sqrt{D \delta^*} \sqrt{\beta S - 1 - \lambda \alpha} , \quad (7)$$

278 which is computed in more details in the Materials and Methods. This expression (7) indicates that  
279 the population composition crucially influences the spreading speed at much lower fractions of  
280 resistant bacteria than the herd immunity threshold (4), where phage expansion stops completely.  
281 Consequently, the resulting plaque radius  $r$  decays with increasing fractions of resistants and  
282 reaches zero at  $H$ . A prediction for  $r$  can be obtained by integrating (7) over time.

283 In our (simplified) model, time-dependence of the speed only enters via the fraction of suscep-  
284 tibles  $S$ , which is assumed to stay at the initial  $S_0$  value until it encounters the epidemic wave of  
285 phages. Furthermore, we use the experimental observation that plaque expansion ceases upon de-  
286 pletion of nutrients, coinciding with a cessation of bacterial growth. This leads to the approximation  
287  $r \approx v T_{\text{depl}}$ , with  $T_{\text{depl}}$  given by Eq. (3). Using this expression we estimated the adsorption constant  
288  $\delta^*$  from the growth experiments as it is difficult in practice to measure on plates. The green line  
289 in Fig 5B is the best fit, yielding the value  $\delta^* = 4.89(\pm 0.19) \cdot 10^{-2}$  bacteria/phage  $h$  for the adsorption  
290 constant.

291 Our results for spatially structured populations allows us to speculate on a general epidemiolog-  
292 ical question: If an infection is not stopped by herd immunity in a partially structured population, by  
293 how much is its spread slowed down? By generalizing (7) we can derive a relative expansion speed,  
294 compared to a fully susceptible population,

$$v_{\text{rel}} = \sqrt{1 - \frac{1 - S}{H}} . \quad (8)$$

295 This expression, (8), is devoid of any (explicit) environmental conditions, which are not already  
296 contained in the herd immunity threshold  $H$  itself. Thus, it could apply to any pathogen-host  
297 system. Ultimately, this relative speed approaches zero with a universal exponent of  $1/2$ , when

the fraction of resistant individuals  $1 - S$  approaches the herd immunity threshold  $H$ . However, a few caveats exist when using (8), as several conditions have to be fulfilled: Obviously, a pathogen is expected not to spread in a population exhibiting complete herd immunity – the relative speed should only hold for populations below the herd immunity threshold. Moreover, if dispersal cannot be described by diffusion, but rather dominated by large jumps (*Hallatschek and Fisher, 2014*), the diffusion approach we used for traveling waves is not applicable, and thus also renders (8) inadequate.

An increase in the number of long range jumps of phages can be considered as a transition between the two cases we treated here – spatially explicit dynamics on plates and completely mixed populations in liquid culture, respectively. Potential long range jumps of phages can be mediated by host cells moving distances that the phages cannot achieve on their own. In such cases, dispersal of the phages is a convolution of movement of their hosts with their own ability to spread locally. These long range jumps would therefore increase the overall expansion speed and area of the epidemic. We expect that in our setup bacterial motility does not substantially contribute to phage spread because (i) bacteria become motile only in late exponential / early stationary phase (*Amsler et al., 1993*) when phage reproduction drops to very low levels, and (ii) the soft agar concentration used in our experiments ( $\approx 0.525\%$ ) effectively blocks bacterial motility (*Croze et al., 2011*). However, we would not expect that long range jumps change the herd immunity threshold  $H(\alpha)$  itself. Spread of pathogens still stops when the fraction of susceptible hosts  $S$  is small such that  $\beta S < 1 + \lambda\alpha$ , and will continue as long as  $\beta S > 1 + \lambda\alpha$  is fulfilled.

## Discussion

The spread of a pathogen may be halted or slowed by resistant individuals in a population and thus provide protection to susceptible individuals. This process, known as herd immunity, has been extensively studied in a wide diversity of higher organisms (*Jeltsch et al., 1997; Mariner et al., 2012; Boven et al., 2008; Meister et al., 2008; Konrad et al., 2012; Wang et al., 2013*). However, the role of this process has largely been ignored in microbial communities. To delve into this we set out to determine under what conditions, if any, herd immunity might arise during a phage epidemic in bacterial populations as it could have profound implications for the ecology of bacterial communities.

We show that herd immunity can occur in phage-bacterial communities and that it strongly de-

328 depends on bacterial growth rates and spatial population structure. Average growth rates of bacteria  
 329 in the wild have been estimated as substantially slower than in the laboratory (generation time is  $\approx$   
 330 6.5 fold longer (*Gibson et al., 2016*)), a condition that we have shown to facilitate herd immunity.  
 331 Furthermore, bacterial populations in the wild are also highly structured, as bacteria readily form  
 332 micro-colonies or biofilms (*Hall-Stoodley et al., 2004*) and grow in spatially heterogeneous environ-  
 333 ments such as soil or the vertebrate gut (*Fierer and Jackson, 2006*), a second condition we have  
 334 shown to facilitate herd immunity. These suggest that herd immunity may be fairly prevalent in low  
 335 nutrient communities such as soil and oligotrophic marine environments.

336 In an evolutionary context, herd immunity may also impact the efficacy of selection as the  
 337 selective advantage of a resistance allele will diminish as the frequency of the resistant allele in  
 338 a population approaches the herd immunity threshold,  $H$ . This has two important implications.  
 339 First, while complete selective sweeps result in the reduction of genetic diversity at linked loci, herd  
 340 immunity may lead to only partial selective sweeps in which some diversity is maintained. Second,  
 341 herd immunity has a potential to generate and maintain polymorphism at immunity loci, as has  
 342 been shown for genes coding for the major histocompatibility complex (MHC) (*Wills and Green,*  
 343 *1995*). Polymorphism in CRISPR spacer contents have been demonstrated in various bacterial (*Tyson*  
 344 *and Banfield, 2008; Sun et al., 2016; Kuno et al., 2014*) and Archaeal (*Held et al., 2010*) populations  
 345 and communities (*Pride et al., 2011; Zhang et al., 2013; Andersson and Banfield, 2008*). While these  
 346 studies primarily explain polymorphisms in CRISPR spacer content as a result of rapid simultaneous  
 347 independent acquisition of new spacers, we suggest that observed polymorphisms may result from  
 348 frequency-dependent selection on resistance loci arising from herd immunity. In such a case, herd  
 349 immunity is likely to maintain existing polymorphism in CRISPR spacer content in  $1 - H$  fraction of  
 350 the population, unless the current major variant goes to fixation due to drift. However, considering  
 351 the large population sizes of bacteria, drift is unlikely to have a strong effect, allowing herd immunity  
 352 to maintain a large fraction of immunity polymorphism.

353 It has also been suggested that herd immunity might favor coexistence between hosts and  
 354 their pathogens (*Hamer, 1906*), which can lead to cycling in pathogen incidence and proportions of  
 355 resistant and susceptible individuals over time, e.g., in measles before the era of vaccination (*Fine,*  
 356 *1993*). This cycling is caused by the birth of susceptible individuals, which, once their proportion  
 357 exceeds the epidemic threshold ( $1 - H$ ), lead to recurring epidemics. CRISPR-based immunity is,  
 358 however, heritable meaning that descendants of resistant bacteria remain resistant. One might



speculate that analogous cycling in phage epidemics may occur if immunity is costly. In turn, a computer simulation study of coevolution of *Streptococcus thermophilus* and its phage found both cycling and stable coexistence of different CRISPR spacer mutants and phage strains (Childs et al., 2014). The extent to which herd immunity facilitates maintenance of CRISPR spacer polymorphism and coexistence with phage requires further experimental and theoretical investigation.

We also developed a mathematical model and show how the herd immunity threshold  $H$  (Eqn. (4)) depends on the phage burst size  $\beta$  and latent period  $\lambda$ , and on the bacterial growth rate  $\alpha$ . This dependence arises as phages have to outgrow the growing bacterial population, as host and pathogen have similar generation times in our microbial setting. In addition to these parameters, we also describe how the speed  $v$  (Eqn. (7)) of a phage epidemic in spatially structured populations depends on phage diffusion constant  $D$ , phage adsorption rate  $\delta^*$ , and the fraction of resistant and susceptible individuals in the population. All of which are likely to vary in natural populations. We also derived the relative speed of spread for partially resistant populations, as measured relative to a fully susceptible population, and show that it can be parametrized solely with the herd immunity threshold  $H$  (Eqn. (8)). This relative speed of the spread of an epidemic should be applicable to any spatially structured host population where the spread of the pathogen can be approximated by diffusion. Both our experiments and the modelling show that even when the fraction of resistant individuals in the population is below the herd immunity threshold the expansion of an epidemic can be substantially slowed, relative to a fully susceptible population.

In conclusion, we have presented an experimental model system and the connected theory that can be usefully applied to both microbial and non-microbial systems. Our theoretical framework can be useful for identifying critical parameters, such as  $H$  (and to some extent  $R_0$ ), from the relative speed of an epidemic expansion in partially resistant populations so long as the process of pathogen spread can be approximated by diffusion. This approximation has been shown to be useful in such notable cases as rabies in English foxes (Murray et al., 1986), potato late blight (Scherf, 1996), foot and mouth disease in feral pigs (Pech and McIlroy, 1990), and malaria in humans (Gaudart et al., 2010).

386 **Materials and Methods**

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
gene ( <i>Streptococcus pyogenes</i> SF370)	cas9	National Center for Biotechnology Information	NCBI:NC_002737.2; gene_ID:901176; RRID:SCR_006472	Gene symbol SPy_1046
strain, strain background ( <i>Escherichia coli</i> )	E. coli K12 MG1655	Own collection	NA	
strain, strain background ( <i>Bacteriophage T7</i> )	E. coli bacteriophage T7	ATCC Collection	ATCC:BAA-1025-B2; RRID:SCR_001672	
recombinant DNA reagent	pCas9	Addgene Vector Database	Addgene:42876; RRID:SCR_005907	pCas9 plasmid was a gift from Luciano Marraffini
recombinant DNA reagent	pCas9T7resistant	this paper	NA	Plasmid derived from pCas9
commercial assay or kit	PureYield Plasmid Miniprep System	Promega	Promega:A1223; RRID:SCR_006724	
chemical compound, drug	Chloramphenicol	Sigma-Aldrich	Sigma-Aldrich:C0378-5G; RRID:SCR_008988	
software, algorithm	PerkinElmer Volocity v6.3		RRID:SCR_002668	Volocity 3D Image Analysis Software
software, algorithm	Fiji v1.0	doi: 10.1038/nmeth.2019	RRID:SCR_002285	Image processing package of ImageJ
software, algorithm	RStudio 1.0.153		RRID:SCR_000432	Software for the R statistical computing
software, algorithm	Python 3.6.3		RRID:SCR_008394	Python programming language
software, algorithm	Model source code	doi: 10.5281/zenodo.1038582	RRID:SCR_004129	Zenodo repository

**Table 1.** Table of key strains, reagents and software used in this study.387 **Experimental methods**388 **Engineering resistance**

389 Oligonucleotides AAACCTTCGGAAGCACTTGTGGAAG and AAAACTTCCACAAGTGCTTCCCGAA were  
 390 ordered from Sigma-Aldrich, annealed and ligated into pCas9 plasmid (pCas9 was a gift from  
 391 Luciano Marraffini, Addgene plasmid #42876) carrying a *Streptococcus pyogenes* truncated CRISPR  
 392 type II system and conferring chloramphenicol resistance. For the detailed protocol see (*Jiang et al.,*  
 393 **2013**). The oligonucleotides were chosen so that the CRISPR system targets an overlap of phage  
 394 T7 genes 4A and 4B. Therefore, the CRISPR system allows the gene 0.7, coding for a protein which  
 395 inhibits the RNA polymerase of the cell, to be expressed before the T7 DNA gets cleaved (*García and*

396 **Molineux, 1995**). The subsequent growth of the cells is halted and phage replication is inhibited.  
 397 The plasmid was electroporated into *Escherichia coli* K12 MG1655 (F- lambda- *ilvG- rfb-50 rph-1*). The  
 398 T7 wildtype phage was used in all experiments.

#### 399 Efficiency of the CRISPR-Cas system

400 Efficiency of the engineered CRISPR-Cas system was tested using the following protocol: Overnight  
 401 cultures grown in LB containing 25  $\mu\text{g/ml}$  chloramphenicol were diluted 1 in 10 in the same medium,  
 402 cells were infected with the T7 phage (MOI 10, 100, and 1000) and incubated for 15 min in 30°C.  
 403 Cells were spun down for 2 min in room temperature at 21130g. Supernatant was discarded and  
 404 the cell pellet was resuspended in 950  $\mu\text{l}$  of 1X Tris-HCl buffer containing 0.4% ( $\approx 227\mu\text{M}$ ) ascorbic  
 405 acid pre-warmed to 43°C and incubated in this temperature for 3 min to deactivate free phage  
 406 particles (**Murata and Kitagawa, 1973**). Cultures were serially diluted and plated using standard  
 407 plaque assay protocol on a bacterial lawn of susceptible cells to detect bursting infected cells. The  
 408 supernatant was tested for free phage particles, which were not detected in the corresponding  
 409 dilutions used for plaque counting. Each experiment was replicated three or four times (MOI 10  
 410 three times, MOI 100 four times and MOI 1000 three times) while samplings from each treatment  
 411 were performed in quadruplicates. The probability that a resistant cell bursts was calculated as a  
 412 ratio of bursting resistant to bursting susceptible cells for each experiment (means of corresponding  
 413 quadruplicates). All LB agar plates and soft agar used throughout this study was supplemented  
 414 with 25  $\mu\text{g/ml}$  chloramphenicol. These CRISPR-Cas system efficiencies at different MOIs were tested  
 415 if they are statistically different from each other using two-tailed unequal variances t-test at 0.05  
 416 confidence level using RStudio (**R Core Team, 2013**).

#### 417 Determining the mean number of phages per cell

418 The cultures that were plated using standard plaque assays in the “Efficiency of the CRISPR-Cas  
 419 system” experiment were also plated on LB agar plates containing 25  $\mu\text{g/ml}$  chloramphenicol to  
 420 determine the number of surviving CFUs. The numbers of bursting and surviving susceptible cells  
 421 were subsequently used to determine the actual mean number of adsorbed phages per cell. The  
 422 fraction of susceptible cells surviving the phage challenge experiment was assumed to correspond  
 423 to the Poisson probability that a cell does not encounter any phage, which was then used to  
 424 determine the mean of the Poisson distribution, which corresponds to the mean number of phages  
 425 per cell.

## 426 Herd immunity in a liquid culture

427 Herd immunity in a liquid culture was tested in 200  $\mu\text{l}$  of LB broth supplemented with 25  $\mu\text{g}/\text{ml}$   
 428 chloramphenicol in Nunclon flat bottom 96 well plate in a Bio-Tek Synergy H1 Plate reader. Bacterial  
 429 cultures were diluted 1 in 1000 and mixed in the following ratios of resistant to susceptible cells:  
 430 50:50, 75:25, 87.5:12.5, 93.75:6.25, 96.88:3.13, 98.44:1.56, 99.22:0.78, 99.61:0.39, 99.8:0.2, 99.9:0.1,  
 431 99.95:0.05, 100:0 %. T7 phage was added at a multiplicity of infection (MOI) of  $\approx 10^{-4}$  ( $\approx 50$  plaque  
 432 forming units (*pfu*) per culture) to resemble an epidemic initiated by the burst size from one infected  
 433 cell and the cultures were monitored at an optical density 600 nm for 18 hours post inoculation (*hpi*).  
 434 Each population composition was replicated 16 times. Herd immunity in diluted LB was measured  
 435 in LB broth mixed with 1X M9 salts in ratios 1:1 (50% LB) and 1:4 (20% LB) using the same protocol  
 436 as for 100% LB broth. Each population composition was replicated 18 times.

## 437 Time-lapse imaging of plaque growth

438 Soft LB agar (0.7%) containing 25  $\mu\text{g}/\text{ml}$  chloramphenicol was melted and 3 *ml* were poured into  
 439 glass test tubes heated to 43°C in a heating block. After the temperature equilibrated, 0.9 *ml* of  
 440 a bacterial culture consisting of resistant and susceptible cells (ratios 10% – 100% of susceptible  
 441 cells, 10% increments) were diluted 1 in 10 and added to the tubes. Then, 100  $\mu\text{l}$  of bacteriophage  
 442 stock, diluted such that there would be  $\approx 10$  plaques per plate, was added to the solution. Tubes  
 443 were vortexed thoroughly and poured as an overlay on LB agar plates containing 25  $\mu\text{g}/\text{ml}$  chlo-  
 444 ramphenicol. The plates were placed on scanners (Epson Perfection V600 Photo Scanner) and  
 445 scanned every 20 minutes in “Wide Transparency mode” for 48 hours in 30°C. A total of 3 scanners  
 446 were employed with a total of 12 plates, plus a no phage control plate and 100% resistant control  
 447 outside the scanners (see Appendix figure 3). No plaques were detected in the 100% resistant  
 448 controls. Time-lapse images were used to calculate the increase of individual plaque areas using  
 449 image analysis software PerkinElmer Volocity v6.3 and Fiji v1.0 (*Schindelin et al., 2012*).

## 450 Bacterial growth on soft agar

451 Growth rate of susceptible bacteria in soft LB agar (0.7%) was measured by sampling from a petri  
 452 dish with a soft agar overlay with bacteria prepared in the same way as the plaque assays except  
 453 an absence of the phage. Sampling was performed in spatially randomized quadruplicates at the  
 454 beginning of the experiment and subsequently after 2, 4, 6, 8, 10, 12, 14, 16, 24, 32, 40, and 48

	<i>Estimate</i>	<i>Units</i>
$\alpha_{\max}$	0.720 ( $\pm 0.011$ )	$[h^{-1}]$
$K_c$	0.257 ( $\pm 0.012$ )	Relative Nutrient Concentration, $N$ $[0 \dots 1]$

**Table 2. Estimated parameters for bacterial growth using Monod kinetics.** Undiluted LB medium ( $N = 1$ ) is assumed to have 15 mg/ml nutrients (10 mg/ml Tryptone, 5 mg/ml yeast extract). The full dataset is shown in Fig 6.

hours using sterile glass Pasteur pipettes (Fisherbrand art.no.: FB50251). Samples were blown out from the Pasteur pipette using an Accu-jet pro pipettor into 1 ml of M9 buffer pre-warmed to 43°C, vortexed for 15 seconds and incubated for 10 minutes in 43°C with two more vortexing steps after 5 and 10 minutes of incubation. Samples were serially diluted and plated on LB agar plates containing 25 µg/ml chloramphenicol. How bacterial densities change over time, measured as CFU/ml, is shown in Fig 6A.

#### Bacterial growth rates in liquid culture

Nutrient-dependent growth rate of susceptible bacteria was measured in Nunclon flat bottom 96 well plate in Bio-Tek Synergy H1 Plate reader in 30°C. Overnight LB cultures were diluted 1:200 in media consisting of LB broth mixed with 1X M9 salts in ratios 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 and 100:0. Final volume was 200 µl. Optical density at 600 nm was measured every 10 min. Every treatment was replicated eight times. Natural logarithm of the optical density values was calculated to determine the growth rate using a maximal slope of a linear regression of a sliding window spanning 90 min.

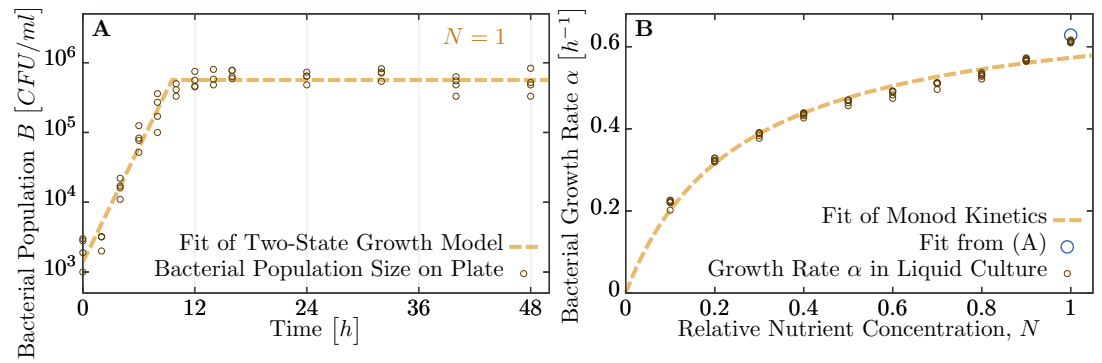
The resulting growth rates for various nutrient concentrations fit well with Monod's growth kinetics,

$$\alpha = \alpha_{\max} \frac{N}{K_c + N} . \quad (9)$$

Results for the two fitting parameters,  $\alpha_{\max}$  and  $K_c$ , are listed in Table 2. The whole dataset, including the fit, is displayed in Fig 6B.

Test for a difference in growth rates of resistant and susceptible bacteria was done in LB broth in the same manner as nutrient-dependent growth rate measurements. Two-sample t-test was performed on acquired growth rate data at 0.05 confidence level using RStudio (*R Core Team, 2013*).

All growth media used in growth rate measurements were supplemented with 25 µg/ml chlo-



**Figure 6. Measuring bacterial growth without phage.** (A) Trajectory of population size on agar plates over time. For modeling, we assume two states of growth (dashed brown curve): first, the bacterial population grows exponential until the time  $T_{\text{depl}}$ , when nutrients are depleted. From this time on, growth rate is assumed to be zero and the population saturates at a maximal size  $B^{\text{final}}$ . Experimental observations fit this proposed growth curve to a very good extent. After all, half of all nutrients are used up in the last generation indicating that the switch between growth and no-growth should be fast. (B) Growth rates of bacteria in diluted medium follow closely Monod's empirical law, given by expression (9). Fit parameters are found to be  $\alpha_{\text{max}} \approx 0.720 \text{ h}^{-1}$  and  $K_c \approx 0.257$  (with the latter in dimensionless units as relative concentrations of standard LB medium), see also Table 2. The data presented in this figure can be found in Figure 6–source data1.

The following source data are available for Figure 6:

Source data 1. Bacterial growth on soft agar plates (tab Fig 6A) and bacterial growth in LB medium of various concentrations (tab Fig 6B).

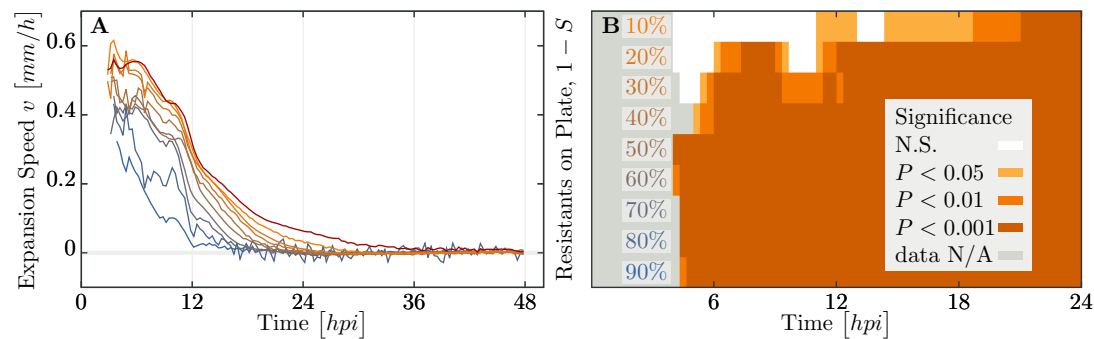
Medium	Rel. Nutr. Conc.	Latent period	Burst size	Burst size/hour
	$N$	$\lambda$ [min]	$\beta$	$\beta/\lambda$ [ $h^{-1}$ ]
LB 0	0.0	101.1 ( $\pm 10.9$ )	3.0 ( $\pm 1.9$ )	1.8 ( $\pm 1.1$ )
LB 20	0.2	43.4 ( $\pm 3.9$ )	12.0 ( $\pm 4.2$ )	16.6 ( $\pm 6.0$ )
LB 50	0.5	40.0 ( $\pm 3.0$ )	35.6 ( $\pm 16.4$ )	53.4 ( $\pm 24.9$ )
LB 100	1.0	36.1 ( $\pm 6.1$ )	85.6 ( $\pm 47.3$ )	142.1 ( $\pm 82.1$ )

**Table 3. Estimated parameters for phage growth.** See also Fig 4A,B.

477 ramphenicol.

478 Phage burst sizes

479 Phage burst sizes in bacteria growing at different growth rates were measured by one-step phage  
 480 growth experiments in LB mixed with 1X M9 salts in the following ratios 0:100, 20:80, 50:50 and  
 481 100:0. The burst sizes were calculated as the ratio of average number of plaques before burst to



**Figure 7. Speed of phage epidemic expansion on bacterial lawns.** (A) Speed of expanding phage epidemics for all population compositions is initially high, before it drops once nutrients are depleted at around 10 hpi (hours post infection). (B) Plaque speed significance. Comparing speeds of plaque spread with the 100% susceptible control. Linear regression of a sliding window spanning 4 hours of the radius sizes was calculated for all individual plaques and all compositions of the populations between  $t_0$  and  $t_{24}$ . Slopes of the linear regressions for all compositions of the populations were compared using a two-sided heteroscedastic t-test against the 100% susceptible dataset. The data presented in this figure can be found in Figure 7-source data1. The following source data are available for Figure 7:

Source data 1. Speed of plaque expansion in populations consisting of varying proportions of resistant to susceptible bacteria.

average number of plaques after burst. Consecutive samplings before and after burst were used for the calculation if they were not significantly different from each other (two sided t-test,  $p > 0.05$ ). All experiments were performed in triplicates.

Phage latent periods

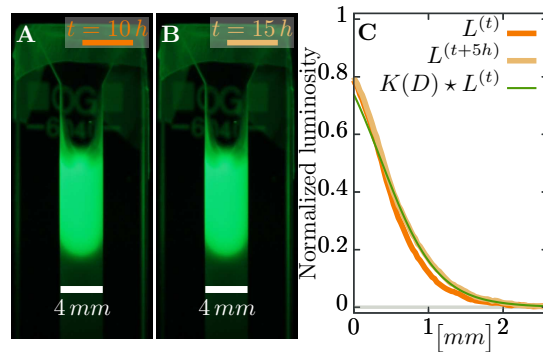
Phage latent periods were determined from the phage burst size experiments as the time interval between the first and the last significantly different consecutive sampling between those used for phage burst size calculations.

Speed of phage expansion

The speed of the phage expansion was measured as difference in radii of plaques over time. Statistical tests allowed to infer that the reduction of expansion speed is significant already for small deviations from the 100% susceptible control experiment, as described and shown in Fig 7.

Phage diffusion in soft agar

Soft M9 salts soft agar (0.5%) was supplemented with SYBR safe staining (final conc. 1%) and poured into glass cuvettes (VWR type 6040-OG) to fill  $\sim 2\text{ cm}$  of the cuvette height. After soft



**Figure 8. Estimating diffusion constant of phages.** (A), (B) Phage are slowly expanding on agar which can be observed via their fluorescence. Pictures are taken 5 h apart. (C) The diffusion constant  $D$  can be estimated as best-fit parameter in a *heat kernel*  $K(D)$ :  $K(D)$  propagates the fluorescence profile  $L^{(t)}$  at time  $t$  forward (via a convolution to “smear” out the signal) to the profile  $L^{(t+\Delta t)}$  at the next measured time point. The difference between the expected change and the actual profile is quantified as total squared deviation, see Eqn. (10), which we minimize to obtain  $D$ . Consequently, we can estimate the diffusion constant as  $D \approx 1.17 \cdot 10^{-2} \text{ mm}^2/\text{h}$ . The green line uses this estimated parameter  $D$  and shows the change between the profile at  $t = 10 \text{ h}$  (orange line) and the profile at  $t = 15 \text{ h}$  (light brown line), assuming diffusive spread of phages. See Materials and Methods for more information.

496 agar solidification, the same stained soft agar was supplemented with T7 phage particles to a  
 497 final concentration  $10^{11}$  pfu/ml and poured on top of the agar without phages. The cuvettes were  
 498 monitored in  $30^\circ\text{C}$  every hour for 40 hours at the SYBR safe emission spectrum peak wave length  
 499  $524 \text{ nm}$  illuminated with the SYBR safe excitation spectrum peak wave length  $509 \text{ nm}$ . The diffusion  
 500 constant was estimated as the best fit parameter for the spread of fluorescent phages through the  
 501 soft agar over time.

502 First we computed the luminosity  $L_i$  of fluorescence (a gray-scale value defined as  $L = 0.2126R +$   
 503  $0.7152G + 0.0722B$  from the RGB image) as average over the width of the cuvette for pixel row  $i$ , and  
 504 corrected the profiles of luminosity  $L_i$  by subtracting the background value. This background value  
 505 was estimated as a linear fit at the end of the profile without phages, where only the gray value  
 506 of the agar was measured. Moreover, in our setup luminosity seems to saturate at values above  
 507  $\sim 0.4$  where it does not have a simple linear dependence on fluorescence anymore: diffusion would  
 508 lead to a decrease of the signal behind the inflection point of the profile and increase after the  
 509 inflection point, but images only show increasing profiles – the bulk density does not decay. Thus,  
 510 any estimate should only take the part of the profile that is below the threshold value of 0.4 into  
 511 account (see Fig 8).



512 The diffusion constant  $D$  itself was estimated as the minimal value of the total squared deviation  
 513 of the convoluted profile  $L^{(t)}$  (at time  $t$ ) with a heat kernel  $K(D)$  compared to the profile  $L^{(t+1)}$  at  
 514 time  $t + 1$ ,

$$D = \left\langle \min_D \sum_i \left( \left( \sum_j \frac{e^{-(i-j)^2/4D}}{\sqrt{4\pi D}} L_j^{(t)} \right) - L_i^{(t+1)} \right)^2 \right\rangle. \quad (10)$$

515 Such a convolution with the heat kernel  $K_{ij}(D) = (4\pi D)^{-1/2} \exp(-(i-j)^2/4D)$  assumes that the only  
 516 change in the profile is due to diffusion for a time span of length 1 with  $i$  and  $j$  indices of pixels.  
 517 Thus, expression (10) estimates the diffusion constant in units of  $\text{pixel}^2/\text{frame}$ , where frame is the  
 518 time difference between two images. Several estimates are averaged over different snapshots in  
 519 the whole experiment that spans  $40 h$  in intervals of  $1 h$  each.

520 The final estimate in appropriate units is

$$D \approx 1.17 (\pm 0.26) \cdot 10^{-2} \text{ mm}^2/h, \quad (11)$$

521 which is in agreement with previous measures of phage diffusion (*Stent and Wollman, 1952; Bayer*  
 522 *and DeBlois, 1974; Briandet et al., 2008*).

## 523 Modelling

### 524 Phage growth

525 In the main text we stated that relevant processes for phages growing on bacteria are given by  
 526 the set of reactions (2). In the following, we will analyze an extended version of our model, which  
 527 takes all these processes into account. We try to justify our approximations and explain the  
 528 reasoning behind leaving parts of the full model out. While reactions for single bacteria or phages  
 529 are inherently stochastic in nature, we assume that the involved numbers are large enough such  
 530 that the dynamics can be described with deterministic differential equations for the populations.  
 531 Furthermore, reaction rates are identified with the inverse of the average time scale of the process.  
 532 Thus, the full model is given by the coupled differential equations,

$$\partial_t B_s = \alpha B_s - A[B_s, P|B_s, B_r], \quad (12a)$$

$$\partial_t B_r = \alpha B_r - A[B_r, P|B_s, B_r] + \rho I_r, \quad (12b)$$

$$\partial_t I_s = A[B_s, P|B_s, B_r] - (1/\lambda) I_s, \quad (12c)$$

$$\partial_t I_r = A[B_r, P|B_s, B_r] - (1/\lambda) I_r - \rho I_r, \quad (12d)$$

$$\partial_t P = (\beta/\lambda)(I_s + I_r) - \sum_{i \in \{s,r\}} A[B_i, P|B_s, B_r] - \sum_{i \in \{s,r\}} A[I_i, P|I_s, I_r], \quad (12e)$$

$$\partial_t N = -\alpha/Y(B_s + B_r). \quad (12f)$$

Both bacterial populations  $B_i$ ,  $i \in \{s, r\}$ , grow with rate  $\alpha$  and decay via adsorption of phages  $A[B_i, P|B_s, B_r]$ , an expression that is specified below. Infected populations  $I_i$  gain numbers by adsorption and decrease via bursting. Resistant bacteria also can recover from their infected state with a recovery rate  $\rho$ . Phages grow by bursting cells, and lose numbers by adsorption to the various bacterial populations. Moreover, explicit dynamics for nutrients is considered, which are drained by each grown cell inversely proportional to the yield  $Y$ , the conversion factor between nutrient concentration and cell numbers. Essentially, this last equation acts as a timer, when we switch from abundant resources to the depleted state: all growth parameters change significantly upon nutrient depletion. Nevertheless, despite the possible deviations, we assume depletion time is given by the simple estimate (3) and only treat the two possible states of abundant and depleted nutrients.

Adsorption of phages, given by the term  $A[B_i, P|B_s, B_r]$ , can be influenced by the whole distribution of populations within the culture. In liquid medium, a common assumption is that this term is proportional to the concentrations of both the phages and cells (*Weitz, 2016*),

$$A[B_i, P|B_s, B_r] = \delta B_i P, \quad (13)$$

with an adsorption constant  $\delta$ . This expression assumes constant mixing of the population and relatively short contact times between phages and bacteria. In general, this system of equations is akin to Lotka-Volterra dynamics, which has been analyzed in great detail, eg. (*Hofbauer and Sigmund, 1998; Nowak, 2006*).

For our ensuing analysis, we neglect the population of infected resistant bacteria  $I_r$ . Upon examining (12d) we find that most cells to leave their infected state by reducing phage DNA via CRISPR/Cas instead of bursting if  $\rho \gg 1/\lambda$ . If furthermore  $\rho \gg \delta P$ , which is true at least in the initial stages of the experiment, essentially all infected resistant bacteria immediately recover from

a phage infection. Consequently, with both conditions, the resistant infected bacteria tend to vanish,  $I_r \rightarrow 0$ , and their dynamics can be neglected. Only in the Appendix (section Simulation of recovery rate) we release this assumption to explicitly cover the full dynamics of (12) in simulations to estimate values for  $\rho$ .

Exponentially growing bacteria lead to double exponential phage growth

For convenience, we transform the populations to the total bacterial density  $B = B_s + B_r$  and introduce the fraction of susceptible cells  $S = B_s/B$ . The crucial assumption for the remainder of this section is that phages burst immediately after infection,  $\lambda = 0$ , such that we can ignore all infected populations. While not a very biological condition, it allows to analyze the model in more detail. Using these simplifications, we obtain

$$\partial_t B = (\alpha - \delta S P) B, \quad (14a)$$

$$\partial_t S = -S(1 - S)\delta P, \quad (14b)$$

$$\partial_t P = (\beta S - 1)\delta B P. \quad (14c)$$

If we assume that in initial stages of phage growth the number of phages is small, ie.  $\delta P \ll \alpha \sim \mathcal{O}(1 \text{ h}^{-1})$ , the dynamics of bacteria and the fraction of susceptibles simplify to  $\partial_t B = \alpha B$  and  $\partial_t S = 0$ . Note that this term  $\delta P$  also occurs in the linear phage dynamics, where it cannot be neglected. In this instance, we need to view  $\delta B$  as a coefficient, which is likely much larger initially. This set of simplified equations can be solved in closed form,

$$S(t) = S_0, \quad (15a)$$

$$B(t) = B_0 \exp(\alpha t), \quad (15b)$$

$$P(t) = P_0 \exp((S_0 \beta - 1)\delta B_0 (\exp(\alpha t) - 1)/\alpha). \quad (15c)$$

The structure of phage dynamics is particularly important here – it exhibits a double-exponential dependence on time  $t$ , which is a very fast, almost explosive, growth. Such double-exponential growth leads to very large population sizes within a short amount of time (but after an extended initial delay). This general behavior of the solution is independent of the actual growth rate of phages, which only has to be positive. Thus, inspecting the exponent in (15c) yields the condition

$$\beta S_0 > 1 \quad (16)$$

for phage growth to be positive. Incidentally, relation (16) is the naive estimate for the number of successful additional infections arising from a single burst. The double exponential time-dependence is central for our arguing that the dynamics can be described by threshold phenomena, given by conditions like (16): Usually, phages are negligible in the dynamics until they grow fast enough to large enough size, such that it is too late for the bacterial population to deal with the overwhelming phage population.

An important question in the context of these solutions is whether nutrients run out before this double-exponential growth of phages occurs. Hence, we compute the time  $T_\delta$  defined as when phages reach a population of  $P(T_\delta) = 1/\delta$  assuming phages grow as (15c) until then. After  $T_\delta$  the assumptions that allowed to obtain (15c) are not valid anymore. Inverting (15c) for time leads to

$$T_\delta = \frac{1}{\alpha} \log \left( 1 + \frac{\alpha \log(1/\delta P_0)}{(\beta S_0 - 1)\delta B_0} \right). \quad (17)$$

Subsequently, we can compare this estimate  $T_\delta$  to the depletion time  $T_{\text{depl}} = (1/\alpha) \log(B_\infty/B_0)$ . When rearranging the inequality  $T_{\text{depl}} > T_\delta$  in terms of the (initial) fraction of susceptibles  $S_0$ , we obtain

$$\beta S_0 > 1 + \frac{\alpha \log(1/\delta P_0)}{\delta(B_\infty - B_0)}. \quad (18)$$

This expression (18) is a condition for phages to reach “large” population sizes before nutrients are depleted by bacteria. The final population density  $B_\infty$  usually fulfills  $\delta B_\infty/\alpha \gg 1$ , such that the correction given by the second term of (18) can be considered small. Thus, if phages grow ( $\beta S_0 > 1$ ), they also grow very fast with a double-exponential time-dependence and reach a considerably large population size before bacteria stop multiplying (for almost all parameter values).

#### Extending analysis to finite burst times

The analysis above only treated the case  $\lambda \rightarrow 0$ . However, we reported that the latency time  $\lambda$  increases significantly when bacterial growth rate  $\alpha$  declines, see Table 3. Considering finite latency times entails dealing with an infected bacterial population  $I$ . (However, we identify  $I \equiv I_s$  and set  $I_r = 0$ .)

To this end, note that we can rearrange (12a) to  $(1 + \lambda \partial_t)I = \lambda \delta S B P$  using the adsorption model in (13). Hence, we can use the differential operator  $(1 + \lambda \partial_t)$  and apply it directly to (12e) to reduce the dependence on  $I$  in this equation at the cost of introducing higher order derivatives. In particular, we obtain

$$\lambda \partial_t^2 P + (1 + \lambda \delta B) \partial_t P + \delta B(\beta S - 1 - \lambda \alpha) P = 0, \quad (19)$$

where we also inserted  $\partial_t B \approx \alpha B$  in the last term, as we aim again for a solution at initial times where  $\delta P \ll \alpha$ . The effects of the limit  $\lambda \rightarrow 0$  are directly observable – no terms are undefined in this limit. In particular, we find that equation (19) and  $\lambda = 0$  lead directly to the dynamics of phages we just analyzed above, obtaining solution (15c).

In principle, (19) is a hyperbolic reaction-diffusion-equation, which is known to occur upon transformation (or approximation) of time-delayed differential equations (*Fort and Méndez, 2002b*). For initial times we can use the solutions  $B(t) = B_0 \exp(\alpha t)$  and  $S(t) = S_0$ . To proceed, we introduce the auxiliary variable

$$z(t) = -\delta B_0 \exp(\alpha t) / \alpha , \quad (20)$$

and assume  $P(z)$  as a function of this new variable  $z$ . We need to transform the differential operators of time derivatives, and obtain  $\partial_t = \frac{\partial z(t)}{\partial t} \partial_z = \alpha z \partial_z$  and  $\partial_t^2 = (\alpha z \partial_z)(\alpha z \partial_z) = \alpha^2 (z \partial_z + z^2 \partial_z^2)$ . Inserting these expressions in (19) and multiplying the whole equation with  $(\alpha^2 \lambda z)^{-1}$  yields the dynamics for phages,

$$0 = z \partial_z^2 P(z) + (b - z) \partial_z P(z) - a P(z) , \quad (21)$$

where the two extant constants are  $a = 1 - (\beta S_0 - 1) / (\lambda \alpha)$  and  $b = 1 + 1 / (\lambda \alpha)$ . Equation (21) is called “Kummer’s equation” with confluent hypergeometric functions  ${}_1F_1$  as solutions (*Abramowitz and Stegun, 1964*, pg. 504),

$$P(z) = A {}_1F_1(a, b; z) + B z^{1-b} {}_1F_1(a - b + 1, 2 - b; z) . \quad (22)$$

The two integration constants  $A$  and  $B$  can be determined via the initial conditions  $P(t = 0) = P_0$  and  $(\partial_t P)(t = 0) = -\delta B_0 P_0$ . Using these conditions, the shape of the solution is again similar to before with  $\lambda = 0$  (double exponential time-dependence), although  $\lambda > 0$  introduces some skew. The most important aspect of this solution (22) is to compute the parameter combination where it switches from a decreasing to increasing function over time. A careful analysis reveals that at the parameter value  $a = 0$  the behavior of the solution changes. Consequently, we find the condition for growing phage populations,

$$\beta S_0 > 1 + \lambda \alpha , \quad (23)$$

which is a non-trivial extension including finite latency times  $\lambda$ .

Note, however, that this relation (23) does not indicate a correction to the general epidemiological parameter  $R_0$ , which can be identified with  $\beta$  in our model. Rather, it shows that a growing bacterial population requires the phage population to grow even faster for a continuous chain of

infections in an epidemic. The term  $\lambda\alpha$  denotes the ratio of generation times of pathogen over host, which in most cases is small and negligible compared to 1. For bacteria and phages, however, which have similar generation times, such a correction is needed to describe the effects of growing host population sizes. In contrast, many other epidemiological models assume the host population size constant and only pathogens are increasing (or decreasing) in number.

While our result (23) suggest that it also should hold in the limit  $\alpha \rightarrow 0$ , it might not necessarily be so. This specific limit is actually quite important for the time when nutrients are depleted in the experiments. However, at several instances in the calculations above we implied a positive  $\alpha > 0$ . The most important of these is the transformation to  $z(t) = -\delta B(t)/\alpha$ , which actually exhibits two problems: dividing by  $\alpha$  should not be allowed and  $B(t)$  is essentially constant and cannot serve as a variable in a differential equation. We also neglected the second term in  $\partial_t B = (\alpha - \delta SP)B$  throughout our calculation. For  $\alpha = 0$  this second term is dominant in bacterial dynamics and would generate non-linear phage dynamics if inserted for  $\partial_t B$  right before stating (19). However, we expect that albeit the process will run *very* slow, and might not be measurable in experiments, the simple condition  $\beta S_0 > 1$  could indicate phage expansion and bacterial decay.

#### Growth of phages on plated bacterial lawn

Spatial modelling of phage expansion has produced several predictions for how plaque radius  $r$  and expansion speed  $v$  are influenced by experimentally adjustable parameters (*Kaplan et al., 1981; Yin and McCaskill, 1992; You and Yin, 1999; Fort and Méndez, 2002a; Ortega-Cejas et al., 2004; Abedon and Culler, 2007; Mitarai et al., 2016*). Here, we try to use a minimal model to estimate these two observables, based on the considerations of previous sections.

One of the main complications arises from the fact that all densities in (12) have a spatial dimension in addition to their time dependence,  $B_i = B_i(\vec{x}, t)$ ,  $i \in \{s, r\}$ . As explained in the main text we only consider phage diffusion, heterogeneities in all other densities are generated only by phage growth. The additional spatial dimension imposes a particular contact network between phages and bacteria, which are not entirely random encounters anymore: One can expect that the size of the bacterial neighborhood  $\hat{B}$  phages are able to explore is only slightly determined by the actual density  $B$ , and can be assumed constant for most of the experiment,  $\hat{B}(B) \approx \text{const}$ . Consequently, the adsorption term can be written in the following way,

$$A[B_i, P|B_s, B_r] = \delta^* \frac{B_i}{B_s + B_r} P, \quad i \in \{s, r\}, \quad (24)$$

which only depends on the *relative* frequencies of bacterial strains. The adsorption constant  $\delta^*$  is both the rate of adsorption and inter-host transit time as determined by the diffusion constant  $D$ . Thus, one can expect the formal dependence  $\delta^* = \delta^*(D, \hat{B}(B))$ . For our particular experimental setup, however,  $\delta^*$  will be treated as a constant. This adsorption term (24) leads to the dynamics of phages

$$\partial_t P = D \nabla^2 P + G[P, S], \quad (25)$$

where we collected all contributions to phage growth in  $G[P, S]$  and added the spatial diffusion term  $D \nabla^2 P$ . For simplicity, we consider only expansion in a single dimension ( $\nabla^2 \equiv \partial_x^2$ ), which has been found to coincide well with the dynamics of plaque growth (Yin and McCaskill, 1992). The growth term for phages is then defined as,

$$G[P, S] = \delta^* (S\beta - 1 - \lambda\alpha) P, \quad (26)$$

where we also consider the correction  $\lambda\alpha$  obtained from the analysis in liquid culture. Due to the different absorption dynamics on plates, however, this correction might be a slight overestimate of the actual term that accounts for bacterial growth. Reaction-diffusion equations similar to (25) have been first analyzed about 80 years ago (Fisher, 1937; Kolmogorov et al., 1937) and since then treated extensively, e.g. (Murray, 2002; van Saarloos, 2003). They admit a traveling wave solution – here, this corresponds to phages sweeping over an uninfected bacterial lawn. In general, the asymptotic expansion speed for the traveling wave solutions is given by the following expression,

$$\begin{aligned} v &= 2\sqrt{D(\partial_P G)[0, S]} \\ &= 2\sqrt{D\delta^*} \sqrt{S\beta - 1 - \lambda\alpha}. \end{aligned} \quad (27)$$

Only the linearized growth rate of phages at very low densities is relevant for the expansion speed,  $\partial_P G[P = 0, S]$ . Thus, the fraction of susceptible individuals  $S$  should be unchanged from its initial value  $S_0$ . It should be noted, that only for  $S_0\beta > 1 + \lambda\alpha$  does Eqn. (27) remain valid, otherwise we have  $v = 0$ . Such a scenario is relevant when nutrients are depleted and phage growth parameters changes to  $\beta_{\text{depl}}$  and  $\lambda_{\text{depl}}$ .

The expression for the expansion speed also shows the need for the spatial adsorption model in (24), in contrast to the liquid case (13). If adsorption would directly depend on the bacterial density  $B$ , the additional linear dependence on  $B$  in (26) would lead to an exponentially increasing speed during the experiment. This is in clear contradiction to experimental observations.

680 The density of phages behind the expanding front is large and as previously noted at large  
 681 MOIs the CRISPR-Cas system fails to provide effective immunity (see section Materials and Methods  
 682 and appendix Infection load and efficiency of the CRISPR/Cas system). However, in comparison to  
 683 an un-structured environment (e.g., liquid) the structured environment effectively limits transit of  
 684 phage from within a plaque to the expanding front: The combined effect of growth and diffusion  
 685 usually generates a much faster expansion of phages during plaque formation, than diffusion alone.  
 686 Only when nutrients are depleted, can pure diffusion processes explain the slow decrease in speed  
 687 observed in experiments (see Fig 7A). Our model assumes a sharp drop to  $v = 0$  at  $T_{\text{depl}}$  for small  $S$ .

688 In order to derive an expression for the plaque radius  $r$ , we integrate the expansion speed (7)  
 689 over time,  $r(t) = \int_0^t dt' v(t')$ . Employing the simplification that only two values of phage growth are  
 690 necessary to describe the dynamics – before  $T_{\text{depl}}$  phages grow normally with  $\beta$  and  $\lambda$ , after  $T_{\text{depl}}$   
 691 phage growth changes to  $\beta_{\text{depl}}$  and  $\lambda_{\text{depl}}$  – we can evaluate the integral for the radius directly, arriving  
 692 at,

$$r(t) = \begin{cases} 2t\sqrt{D\delta^*}\sqrt{S\beta - 1 - \lambda\alpha}, & 0 < t < T_{\text{depl}}, \\ 2\sqrt{D\delta^*}\left(T_{\text{depl}}\sqrt{S\beta - 1 - \lambda\alpha} + (t - T_{\text{depl}})\sqrt{S\beta_{\text{depl}} - 1}\right), & T_{\text{depl}} < t. \end{cases} \quad (28)$$

693 Using this expression we estimated the adsorption constant  $\delta^*$  from the growth experiments as  
 694 it difficult to measure in practice. This estimate is done for radii exactly at the time of nutrient  
 695 depletion  $T_{\text{depl}}$ , and excluding the control experiment with only susceptible cells.

696 Predictions of our model show a discrepancy from experimental results on plates after depletion.  
 697 We independently estimated  $\beta_{\text{depl}} = 3.0$ , which results in  $H_{\text{depl}} = (\beta_{\text{depl}} - 1)/\beta_{\text{depl}} \approx 0.67$ . Thus, all  
 698 experiments with  $S > 0.33$  should exhibit expanding plaques after nutrients are depleted. In the  
 699 experimental setup plaques stop expanding in all mixtures of resistant to susceptible cells ( $S \leq 0.9$ ),  
 700 which would correspond to  $\beta_{\text{depl}} < 1.1$ . This value is, however, still within experimental accuracy of  
 701 our estimates of  $\beta_{\text{depl}}$ .

## 702 Acknowledgments

703 We are grateful to Remy Chait for his help and assistance with establishing our experimental setups  
 704 and to Tobias Bergmiller for valuable insights into some specific experimental details. We thank  
 705 Luciano Marraffini for donating us the pCas9 plasmid used in this study. We also want to express  
 706 our gratitude to Seth Barribeau, Andrea Betancourt, Călin Guet, Mato Lagator, Tiago Paixão and  
 707 Maroš Pleška for valuable discussions on the manuscript. Finally, we would like to thank the *eLife*



708 editors and reviewers for their helpful comments and suggestions.

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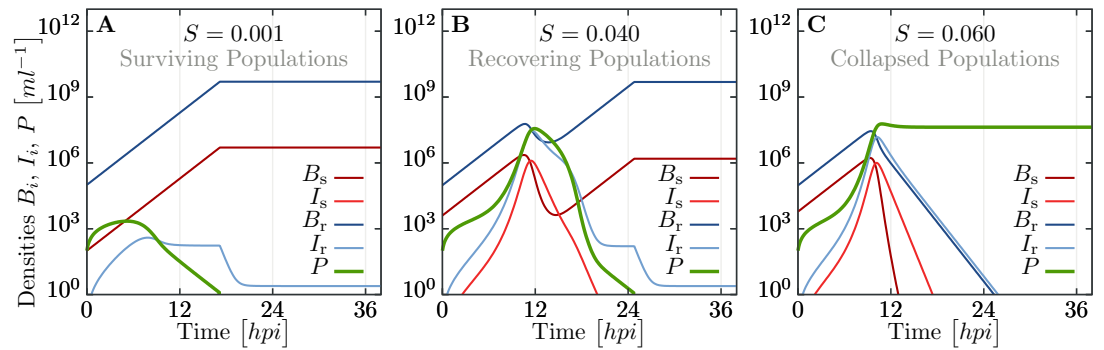
## 900 **Appendix: Additional theoretical considerations**

### 901 **Simulation of recovery rate**

902 Throughout the main text we assumed that resistant bacteria are completely immune to phage  
 903 infection as their CRISPR/Cas system immediately kills adsorbed phages. However, experimental  
 904 observation suggest that for fractions close to what we predicted as herd immunity threshold, *all*  
 905 bacteria eventually die. Thus, in the following section we use numerical simulations to investigate  
 906 the full set of equations (12), with a particular focus on the question why the whole bacterial  
 907 population goes extinct. As it turns out, this requires using finite values for the recovery rate  $\rho$   
 908 (instead of the  $\rho \rightarrow \infty$  approximation employed previously).

909 A major difficulty in analyzing the full model (12) is finding appropriate parameter values.  
 910 In particular, we need values for the adsorption constant  $\delta$ , the recovery rate  $\rho$  and the yield  
 911 coefficient  $Y$ . Undiluted LB medium is known to support a population of  $5 \cdot 10^9$  cells/ml. Thus  
 912 one can easily estimate  $Y$  as the inverse of this number, when nutrients are measured in units  
 913 of relative concentrations of LB, which we already used throughout this publication (undiluted  
 914 medium corresponds to  $N = 1$ ). Parameter scans in simulations reveal that the actual value of  
 915 the adsorption constant  $\delta$  does usually not influence the actual outcome (collapsed or surviving  
 916 bacterial population), it only adjusts time scales. However, deviations in time scales are insignificant,  
 917 even when  $\delta$  is changed by orders of magnitude,  $\delta \sim \mathcal{O}(10^{-6} \dots 10^{-8})$ . They are roughly an hour or  
 918 less, which is small compared to the expected duration of the experiment that lasts a few hours. For  
 919 definiteness, we use the value of  $\delta = 10^{-7} \text{ h}^{-1}$  for our simulations. That the value of the adsorption  
 920 constant has only a minor impact on phage growth on bacterial cultures, is also in line with previous  
 921 findings (*Mitarai et al., 2016*).

922 The most elusive parameter is the recovery rate  $\rho$ . A first indication of the value of  $\rho$  can be  
 923 drawn from our experiments on bursting resistant cells, summarized in Fig 2. As the probability  
 924 for bursting resistant cells is 3 orders of magnitude smaller than for susceptible bacteria, we can  
 925 use  $1/\lambda \sim \mathcal{O}(1)$  to estimate  $\rho \sim \mathcal{O}(10^3)$ . However, our results also indicate that recovery via the  
 926 CRISPR/Cas system heavily depends on MOI, implying that  $\rho$  depends on the actual densities of



**Appendix figure 1. Simulated trajectories for all populations in liquid culture for the extended model, including infected and recovering bacteria.** Trajectories are obtained by numerically integrating equations (12), using parameters listed in Table 4 and additionally  $N = 1$ ,  $Y = 2 \cdot 10^{-10} \text{ cells}^{-1}$ ,  $\delta = 10^{-7} \text{ h}^{-1}$  and  $\rho = 1.5 \text{ h}^{-1}$ . (A) For population compositions with a large majority of resistant cells ( $S = 10^{-3}$ ), phages get wiped out fast. (B) For intermediate  $S$  (close to parameters where we observe both, collapsed and surviving, populations, see Fig 3), the populations exhibit a complex, non-monotonic trajectory. After fast initial growth of phages, bacterial populations decay but ultimately can recover. (C) If the fraction of susceptibles is too large ( $S = 0.06$ ), the whole bacterial population is infected and succumbs to the overwhelming phage infection. See supporting text for more detailed information.

927 phages and bacteria. Nevertheless, as experimental determination of recovery is complicated, even  
 928 more so determining a functional dependence on dynamically changing densities  $B$  and  $P$ , we  
 929 assume that  $\rho$  is constant.

930 We ran parameter sweeps in simulations and compared the outcome – collapsed or surviving  
 931 bacterial populations – to the observed experimental results (see Fig 3). The best agreement  
 932 of simulations and experiments was reached with  $\rho \sim \mathcal{O}(1)$ . Lower values of  $\rho$  do not allow the  
 933 resistant population to recover from phage infection, while for larger values of  $\rho$ , phages are drained  
 934 from the culture very fast. Such a small value of  $\rho$  is most likely related to the recovery at very large  
 935 MOI, when the densities involved in the dynamics are large, which dominate the overall observed  
 936 dynamics. At this time phages repeatedly infect the same bacteria and their CRISPR/Cas immune  
 937 system cannot deal with such an infection load (or only too slow). Thus, we can argue that our final  
 938 choice  $\rho = 1.5 \text{ h}^{-1}$  is the recovery rate when the CRISPR/Cas system is heavily stressed, which is  
 939 comparable to the actual burst rate  $1/\lambda$  for phages.

940 In Appendix figure 1 we show three exemplary sets of trajectories for bacteria and phage. For  
 941 a tiny fraction of susceptibles,  $S = 10^{-3}$ , which is well below the herd immunity threshold (see  
 942 Fig 3), phages do not thrive on the limited number of favorable hosts and decay fast after a slight



increase initially. For intermediate fractions of susceptibles,  $S = 0.04$ , we observe more complex, non-monotonic trajectories of bacterial populations. For such values of  $S$  we also observe mixed outcomes in experiments, see Fig 3. When  $S$  is increased further ( $S = 0.06$ ), enough susceptible bacteria exists to produce enough phages and ultimately the whole bacterial population goes extinct.

The purpose of the extended model in this section was to justify the fact that phages can wipe out the whole bacterial population, which was not possible in the simplified model used in the main text. There, the resistant bacterial population was basically unaffected by phages and just acted as “sink” for phages. However, also in this extended model, we see a very similar behavior in terms of the threshold phenomena reported earlier in the manuscript.

### Infection load and efficiency of the CRISPR/Cas system

In the section [Modelling](#) we showed that positive phage growth leads eventually to a very fast increase in the phage population, that occurs before nutrients are depleted (for almost all realistic parameters). This behavior of the dynamics was also observed in the extended simulation model presented in the last section. Moreover, as a condition we used that the phage population reaches a size  $P \sim 1/\delta$ , which is after all arbitrary – it only determines if we can employ useful simplifications and approximations to model equations. However, simulation results presented in the last section

Parameter		Value	Units	Comment
Bacterial growth rate	$a$	0.63	1/h	Table 2, Fig 6
Yield	$y$	$2 \cdot 10^{-10}$	1/cell	Measured in fractions of $N$
Recovery rate	$\rho$	1.5	1/h	See this appendix
Adsorption constant	$\delta$	$10^{-7}$	1/(h cell)	See this appendix
Diffusion constant	$D$	$1.17 \cdot 10^{-2}$	$mm^2/h$	See Methods
Burst size	$\beta$	85.6	phages/cell	Table 3, Fig 4
Latency time	$\lambda$	0.60	h	Table 3, Fig 4
Initial bacterial population	$B_0$	$10^5$	cells	
Initial phage population	$P_0$	10	phages	

**Appendix 0 Table 4. Parameters used in simulations shown in Appendix figure 1.**

960 indicate that the bacterial population starts to decay soon after such a threshold  $P \sim 1/\delta$  is  
 961 exceeded.

962 In order to proceed, we investigate the system at time  $T_\delta$  further. We assume that the phage  
 963 population is large enough that it will not be degraded by the CRISPR/Cas immune system. The  
 964 threat to immediate phage extinction is low at this point. The actual equations are hard to solve  
 965 directly, hence we revert to simple balance equations, ignoring the dynamical component. Specif-  
 966 ically, we compare the number of (present and eventually produced) phages to the number of  
 967 infections needed to wipe out the whole population. To incorporate the effects of the bacterial  
 968 immune system in resistant bacteria, we assume that they need  $M > 1$  infections before they burst  
 969 and produce only  $\kappa\beta$  phages, which reduces the burst size by a (yet unspecified) factor  $0 < \kappa < 1$ .  
 970  $\kappa = 1$  implies that resistant cells produce the same number of phages as susceptible cells, while  
 971  $\kappa = 0$  indicates only cell death. Combining these considerations yields

$$\underbrace{1/\delta}_{\text{phages present}} + \underbrace{\beta S_0 B(T_\delta)}_{\text{phage production } B_s} + \underbrace{\kappa\beta(1 - S_0)B(T_\delta)}_{\text{phage production } B_r} > \underbrace{S_0 B(T_\delta)}_{\text{infections } B_s} + \underbrace{M(1 - S_0)B(T_\delta)}_{\text{infections } B_r}, \quad (29)$$

972 where the left side indicates the total number of phages, while the right side indicates the number of  
 973 necessary infections to kill all bacteria. The number of bacteria  $B(T_\delta)$  can be estimated by inserting  
 974 the time  $T_\delta$  from (18) into the exponential growth (15b). Subsequently, we can rearrange (29),  
 975 obtaining a bound on  $M$ :

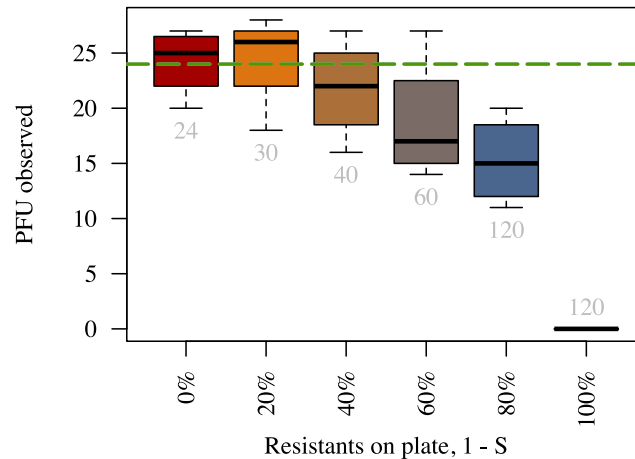
$$M < \frac{1/\delta B(T_\delta) + S_0(\beta - 1)}{1 - S_0} + \kappa\beta. \quad (30)$$

976 The first term  $1/\delta B(T_\delta)$  indicates the ratio of phages to bacteria at time  $T_\delta$ , and can be considered  
 977 small for non-extremal parameters compared to the other terms. This fact justifies our assumption  
 978 that the actual value of  $\delta$  is not crucial. This number  $M$  might allow some insight into the effective-  
 979 ness of the CRISPR/Cas immune system. For a fraction of susceptibles  $S = 0.03$ , which corresponds  
 980 to the minimal value where we observe only collapsed bacterial populations in undiluted LB medium  
 981 (see Figs 3 and 4), we would obtain the relation  $M \lesssim 3 + 86\kappa$ . Thus, each resistant bacterial cell could  
 982 degrade up to  $\mathcal{O}(10^1 \dots 10^2)$  phages before their CRISPR/Cas system cannot cope with the infection  
 983 load anymore.

## Appendix: Supplementary results

### Reduction in number of plaques in spatially structured populations

The reduction in the number of plaques with increasing proportions of resistant bacteria is shown in Appendix figure 2.



### Appendix figure 2. Number of plaques declines faster than proportionally to the fraction of resistant

**bacteria.** Number of plaque forming units observed on a plate (y-axis) for different proportions of resistant bacteria (x-axis). Grey numbers below each boxplot indicate the average number of phages inoculated in the respective treatment. The numbers of phages inoculated were chosen to retain the expected number of plaques on the plate (green dashed line) as in the 0% resistant treatment (red boxplot). Plates were prepared using identical procedure as in Time-lapse imaging of plaque growth (see Materials and Methods). The data presented in this figure can be found in Appendix figure 2–source data1.

The following source data are available for Appendix figure 2:

Source data 1. Measurements of plaque numbers in populations consisting of varying proportions of resistant to susceptible bacteria.

## Appendix: Additional information on experimental setup

Our experimental setup for the scanner system is shown in Appendix figure 3.

## Appendix: Source data and code

Time-lapse images of spread of T7 phage epidemics in Escherichia coli spatially structured populations are available on the Dryad Digital Repository: <https://doi.org/10.5061/dryad.42n44>. Source



**Appendix figure 3. Image of the scanner system.** Photograph of the scanner system used for time-lapse imaging of phage spread in spatially structured bacterial populations. Three scanners (Epson Perfection V600 Photo Scanner) simultaneously scanned 12 plates in total every 20 minutes in 30°C for 48 hours per experiment.

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code of the model presented here is available on GitHub (<https://github.com/lukasgeyrhofer/phagegrowth>) (Payne *et al.*, 2018) and its archived version is accessible through Zenodo: <https://dx.doi.org/10.5281/zenodo.1038582>.